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EXAMINER	
FOSTER, CHRISTINE E	
ART UNIT	**PAPER NUMBER**
1641	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/507,479	REGINSTER ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 June 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7* is/are rejected.

7)☒ Claim(s) *1* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 September 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

1. Applicant's amendment, filed 6/4/07, is acknowledged and has been entered. Claims 1-7 have been amended. Claims 8-26 were cancelled. Claims 1-7 are currently pending and under examination.

Objections/Rejections Withdrawn

2. The objection to the specification has been obviated by the amendments thereto.

3. The objections to claims 2-3, 5, and 9 as set forth in the previous Office action have been withdrawn in response to the amendments and in light of the cancellation of claim 9.

4. The rejections of claims 1-9 under 112, 2nd paragraph has been withdrawn in response to the amendments to remove the term "characteristic of" and in light of the cancellation of claim 9.

5. The rejections of claims 1-6 and 8 under 35 USC 102(b) as being anticipated by Ter Steege et al., of claims 1, 5 and 8 under 35 USC 102(b) as being anticipated by Mikotor et al., and of claims 1 and 5-7 under 35 USC 102(b) as being anticipated by Paik et al. have been withdrawn in response to the amendments to include elements of claim 9 (now cancelled) into the independent claim.

Oath/Declaration

6. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

> The oath or declaration is defective because:
> It does not identify the city and either state or foreign country of residence of each inventor. The residence information may be provided on either an application data sheet or supplemental oath or declaration.

Specifically, the residence address for Inventor Stephan Christgau is not listed on the oath or on an application data sheet.

Applicant is reminded that this deficiency in the oath or declaration **may be corrected with an application data sheet** in accordance with § 1.76. See MPEP 603.

Priority

7. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Specifically, it is noted that the Oath refers to **U.S. Provisional Application 60/363,925**, filed on 03/13/2002. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

8. If applicant desires to claim the benefit of a prior-filed application under 35 U.S.C. 119(e), a specific reference to the prior-filed application in compliance with 37 CFR 1.78(a) must be included in the first sentence(s) of the specification following the title or in an application data sheet. For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of the applications.

If the instant application is a utility or plant application filed under 35 U.S.C. 111(a) on or after November 29, 2000, the specific reference must be submitted during the pendency of the application and within the later of four months from the actual filing date of the application or sixteen months from the filing date of the prior application. If the application is a utility or plant

application which entered the national stage from an international application filed on or after November 29, 2000, after compliance with 35 U.S.C. 371, the specific reference must be submitted during the pendency of the application and within the later of four months from the date on which the national stage commenced under 35 U.S.C. 371(b) or (f) or sixteen months from the filing date of the prior application. See 37 CFR 1.78(a)(2)(ii) and (a)(5)(ii). This time period is not extendable and a failure to submit the reference required by 35 U.S.C. 119(e) and/or 120, where applicable, within this time period is considered a waiver of any benefit of such prior application(s) under 35 U.S.C. 119(e), 120, 121 and 365(c). A benefit claim filed after the required time period may be accepted if it is accompanied by a grantable petition to accept an unintentionally delayed benefit claim under 35 U.S.C. 119(e), 120, 121 and 365(c). The petition must be accompanied by (1) the reference required by 35 U.S.C. 120 or 119(e) and 37 CFR 1.78(a)(2) or (a)(5) to the prior application (unless previously submitted), (2) a surcharge under 37 CFR 1.17(t), and (3) a statement that the entire delay between the date the claim was due under 37 CFR 1.78(a)(2) or (a)(5) and the date the claim was filed was unintentional. The Director may require additional information where there is a question whether the delay was unintentional. The petition should be addressed to: Mail Stop Petition, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

If the reference to the prior application was previously submitted within the time period set forth in 37 CFR 1.78(a), but not in the first sentence(s) of the specification or an application data sheet (ADS) as required by 37 CFR 1.78(a) (e.g., if the reference was submitted in an oath or declaration or the application transmittal letter), and the information concerning the benefit claim was recognized by the Office as shown by its inclusion on the first filing receipt, the

petition under 37 CFR 1.78(a) and the surcharge under 37 CFR 1.17(t) are not required. Applicant is still required to submit the reference in compliance with 37 CFR 1.78(a) by filing an amendment to the first sentence(s) of the specification or an ADS. See MPEP § 201.11.

Claim Objections

9. Claim 1 is objected to because of the following informalities:

10. Claim 1 recites "an amino acid sequence in a free fragment form **comprising sequence**" in lines 2-3 and 4-5, which is objected to for grammatical reasons. It appears that an article such as "the" is required between the words "comprising" and "sequence".

Claim Rejections - 35 USC § 112

11. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

12. Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

New Matter

13. Claim 1 as amended in the Reply of 6/4/07 recites a method of detecting "an amino acid sequence **in a free fragment form** comprising sequence HRGYPGLDG (SEQ ID NO: 1) in

which the amino acid residue Y is nitrated tyrosine; **or an amino acid sequence in a free fragment form comprising sequence HRGYPGLDG (SEQ ID NO: 1) and a nitrated tyrosine**". Applicant's Reply does not specifically indicate where support may be found for the noted limitations, and support could not be found in the specification and claims as originally filed, for the following reasons.

14. With respect to the limitation that the detected amino acid sequence is in a "**free fragment form**", the only reference to a "free" fragment form in the specification was found at page 30, lines 5-6, where it is stated that "[t]he nitrosylated sequence is recognized only in the form of free fragments". However, this disclosure does not adequately support the claimed methods since it refers to a specific example and is presented in the context of describing a property of a specific antibody, the D33 antiserum (see page 29, line 28 to page 30, line 6). Thus, the disclosure is not commensurate with the scope of the claims, which are not limited to methods using D33 antibody or even to methods that involve antibody detection. There is no disclosure at all relating to detection of sequences "in a free fragment form" by other detection methods that would be encompassed by the claim, e.g. HPLC.

Furthermore, the mention of "free" fragments in the Example on page 30 is in distinguishing "free fragments" from "full length protein" (lines 1-6). However, the specification does not clearly define or set forth the metes and bounds of the term "free" in the context of the claimed invention, such that the term "free fragment form" would read on subject matter beyond that disclosed in the specification.

For example, the term "free" might also refer to the fragment being present in an unbound form, as opposed to being attached to a binding partner. In addition, given that the SEQ

ID NO:1 sequence is a fragment of collagen, the claim might also be interpreted as meaning that the amino acid sequences detected are in the unwound or linear conformation, as opposed to the wound or triple-helix conformation of native collagen. The specification does not describe such embodiments as would be reasonably encompassed by the claims. For these reasons, the claimed methods of detecting amino acid sequences "**in a free fragment form**" go beyond the scope of the disclosure and claims as originally filed.

15. With respect to the limitation that the amino acid sequence detected may be **"in a free fragment form comprising sequence HRGYPGLDG (SEQ ID NO: 1) and a nitrated tyrosine**", support could not be found for the noted limitation and Applicant's reply does not indicate where support may be found.

It is noted that this limitation introduces the concept of detecting a genus of amino acid sequences that include the (non-nitrosylated) sequence HRGYPGLDG (SEQ ID NO: 1) as well as a nitrated tyrosine residue *elsewhere in the sequence*. Support for this concept could not be found in the specification. The specification discloses with particularity the specific amino acid sequence of HRGYNO$_2$PGLDG, where the Y-NO$_2$ residue is nitrated tyrosine (see at page 7, lines 23-30; page 12, lines 24-30; and Example 1). Although the specification describes raising context-dependent antibodies against the specific amino acid sequence of HRGYNO$_2$PGLDG, it does not describe any means of detecting amino acid sequences in free fragment form that comprise HRGYPGLDG (without the nitrotyrosine) but which have a nitrotyrosine at some other portion of the amino acid sequence.

In particular, the claims encompass detection of the claimed sequences by immunoassay using an antibody that is reactive with nitrated tyrosine in a context-dependent manner (see claim

3). This means that the epitope bound by the antibody is not simply a nitrated tyrosine residue, but a nitrated tyrosine residue surrounded by particular other residues. However, in the case of amino acid sequences "comprising sequence HRGYPGLDG (SEQ ID NO: 1) and a nitrated tyrosine" the context of the nitrated tyrosine, and therefore the epitope bound by the antibody, is not known. As such, one skilled in the art cannot envisage possession of methods of detecting such sequences since neither the epitope bound by the antibody nor the specific antibody that could be used to detect such sequences have been described. Applicant is attempting to reference an unknown by reference to another unknown.

With respect to claim 4, which now claims methods of detecting (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue since although the epitope recognized by the first antibody is recited (nitrated tyrosine), the second antibody epitope is not known. Further, even if the second antibody epitope were known, one skilled in the art cannot envisage how an anti-nitrated tyrosine antibody and a second antibody to another sequence could be used to detect those particular sequences that comprise HRGYPGLDG. Since in this case neither antibody is recited as binding to the HRGYPGLDG sequence, there is no basis for selective detection of sequences that include HRGYPGLDG.

In summary, the genus of amino acid sequences that contain HRGYPGLDG without the nitrated form of the Y residue, yet which do contain a nitrotyrosine (i.e., in some other portion of the amino acid sequence) is not adequately described in the specification. Furthermore, one skilled in the art would not envisage possession of the currently claimed methods of detecting such sequences, given that the specification directs the skilled artisan to raise context-independent antibodies against nitrotyrosine in the context of HRGYNO$_2$PGLDG sequence but

does not describe detection of fragments that contain HRGYPGLDG as well as a nitrotyrosine residue at some other unspecified location.

16. Claims 3-4 as amended in the Reply of 6/4/07 recite an antibody that "is specifically reactive with said nitrated tyrosine in the context that **said amino acid sequence is specific to and uniquely identified with a specific protein**". This terminology was not found in the specification or claims as originally filed. Applicant's reply indicates that this terminology replaces the term "characteristic of" but does not specifically indicate where support may be found in the specification for such terminology in the specification (see Reply, page 7).

The disclosure of amino acid sequences that are "characteristic of" does not adequately support the currently claimed amino acid sequences that are "specific to and uniquely identified with a specific protein". This conveys a difference in scope, since a sequence "characteristic of" a protein could simply refer in general to a sequence that is found in that protein. By contrast, the recitation of a sequence being "specific to and uniquely identified with a specific protein" indicates a more specific meaning, e.g. that the sequence is only found in that protein. The general reference to a sequence "characteristic of" a specific protein does not adequately convey evidence of possession of amino acid sequences that are "specific to and uniquely identified with" a specific protein.

17. Similarly, claim 5 recites that the amino acid sequence that is detected is "specific to and uniquely identified with a mammalian protein", which is not adequately supported by the disclosure of an amino acid sequence that is "characteristic of" a specific protein.

Written Description

18. The claims are drawn to methods of detecting an "**amino acid sequence in free fragment form comprising [the] sequence HRGYPGDLG (SEQ ID NO: 1) in which the amino acid residue Y is nitrated tyrosine; or an amino acid sequence in a free fragment form comprising [the] sequence HRGYPGLDG (SEQ ID NO: 1) and a nitrated tyrosine**".

In other words, the method detects either (a) sequences comprising HRGY-NO_2-PGLDG or (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue at some other position in the sequence.

The specification discloses that the nitrated amino acid sequences may be detected by immunoassay, in which an antibody is employed which is reactive with the nitrated amino acid residue. It is noted the claims currently encompass both "context-independent" and "context-dependent" antibodies. "Context-independent" antibodies are those that specifically bind to (for example) nitrotyrosine *per se*, regardless of the identity of the surrounding amino acids. By contrast, "context-dependent" antibodies would only recognize nitrotyrosine when it is in the context of other specific amino acids. See the specification, for example at page 4, line 25 to page 5, line 8. In other words, the epitope recognized by "context-independent" antibodies is nitrotyrosine itself (or another nitrated amino acid residue), while the epitope recognized by "context-dependent" antibodies is defined not only by the nitrotyrosine residue but also includes surrounding amino acids.

The courts have stated that "as long as an applicant has disclosed a "fully characterized antigen," either by its structure, formula, chemical name, or physical properties, or by depositing the protein in a public depository, the applicant can then claim an antibody by its binding affinity to that described antigen." *Noelle v. Lederman*, 355 F.3d at 1349 (Fed. Cir. 2004, emphasis in the

original). Although *Noelle* relates to antibodies *per se* and not to detecting methods using such antibodies, the holdings of those cases are also applicable to claims such as those at issue here. A disclosure that does not adequately describe a product itself logically cannot adequately describe a method of using that product.

Applicant has failed to provide written description for the currently claimed genus of detection methods using either "context-dependent" or "context-independent" antibodies.

In the case of "context-independent" detection of nitrotyrosine-containing sequences using antibodies that recognize nitrotyrosine *per se,* one skilled in the art would accept that an adequate description of the specific antigen epitope (nitrotyrosine) would put an inventor in possession of antibodies that bind to nitrotyrosine in a context-independent manner, given that production of antibodies against a well-characterized antigen was conventional at the time of filing.

However, Applicant has not described how *context-independent* antibodies that recognize nitrotyrosine in any context may be used to detect the specific amino acid sequences claimed, such as those that comprise HRGY-NO_2-PGLDG or HRGYPGLDG, since by definition, antibodies that are context-independent would bind to all nitrotyrosine-containing sequences, and thus would not specifically detect certain sequences.

For example, in the case of the claimed methods detecting (a) sequences comprising HRGY-NO_2-PGLDG, Applicant has not described how a context-independent antibody that binds to all nitrated tyrosine residues could be used to detect sequences that include this specific epitope, since by definition, antibodies that are context-independent would bind to all

nitrotyrosine-containing sequences, and thus would not *specifically* react with those sequences that comprise HRGY-NO_2-PGLDG.

Similarly, Applicant has not demonstrated evidence of possession of methods of detecting amino acid sequences in free fragment form that (b) comprise the sequence HRGYPGLDG and a nitrated tyrosine residue (i.e., elsewhere in the sequence), since although a context-independent anti-nitrotyrosine antibody may bind to such sequences, it would also bind to all other nitrotyrosine-containing sequences. This would not result in preferential detection of amino acid sequences in free fragment form that comprise the sequence HRGYPGLDG and a nitrated tyrosine residue as claimed. See also the new matter rejection above.

With respect to the two-antibody methods of claim 4, which involve a first context-independent antibody (e.g., anti-nitrotyrosine antibody) followed by a second antibody that is specific for "an amino acid sequence which is specific for and uniquely identified with a specific protein", Applicant has failed to provide adequate written description of such methods since the "amino acid sequences that are specific for and uniquely identified with a specific protein" (i.e., the epitope recognized by the second antibody) lacks written description.

Such "specific for and uniquely identified" amino acid sequences presumably refer to other, non-nitrated portions of the fragment sequence to be detected, yet Applicant has described **no** such amino acid sequences with any particularity. The characteristics of the genus are not known. Put another way, the epitope recognized by the second antibody is not known. Applicant has also not described any second antibodies that recognize such sequences. Neither the recited "amino acid sequences… specific for and uniquely identified with a specific protein" nor the

"second antibody" are adequately described in the specification. Applicant is attempting to describe an unknown by reference to another unknown.

For these reasons, Applicant has not demonstrated evidence of possession of methods of detecting the claimed sequences using a first context-independent anti-nitrated tyrosine antibody and a second antibody that binds to "amino acid sequences that are specific for and uniquely identified with a specific protein" since the epitope recognized by the second antibody is not known.

Even if the second antibody epitope were known, Applicant has not described how an anti-nitrated tyrosine antibody and a second antibody to some other sequence could be used to detect sequences comprising (a) HRGY-NO_2-PGLDG. Since in this case neither of the two antibodies is recited as binding to the HRGY-NO_2-PGLDG sequence, there is no basis for selective detection of sequences that include HRGY-NO_2-PGLDG.

Similarly, Applicant has also not demonstrated evidence of possession of methods of detecting (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue since although the epitope recognized by the first antibody is recited (nitrated tyrosine), the second antibody epitope is not known. Further, even if the second antibody epitope were known, Applicant has not described how an anti-nitrated tyrosine antibody and a second antibody to another sequence could be used to detect those particular sequences that comprise HRGYPGLDG. Since in this case neither antibody is recited as binding to the HRGYPGLDG sequence, there is no basis for selective detection of sequences that include HRGYPGLDG.

Turning now to the use of context-dependent antibodies (as in claim 3), the claims now recite methods of detecting (b) sequences comprising HRGYPGLDG and a nitrated tyrosine

residue using a context-dependent anti-nitrotyrosine antibody. However, Applicant has not disclosed what context the nitrated residue would be found in, i.e. the identity of the surrounding residues. The antibody only binds to nitrated tyrosine in a specific context, yet that context is not described. The reference to nitrated tyrosine alone is not enough information to identify the antibody epitope. As such, neither the epitope recognized by the context-dependent antibody nor the antibody itself has been described. Applicant is attempting to reference an unknown by reference to another unknown.

Although claim 1 now recites that the amino acid sequences detected may (a) comprise the sequence HRGYPGLDG where the Y is nitrated tyrosine, Applicant has not demonstrated evidence of possession of all methods of detecting such sequences. In particular, Applicant has not disclosed any methods of detecting fragments that comprise this particular sequence except by the use of context-dependent antibodies that recognize the nitrated tyrosine in the context of the HRGYPGLDG epitope. Such a disclosure is not commensurate with the scope of the claims.

Furthermore, although claim 1 as amended recites detection of an amino acid sequence "in free fragment form", the evidence of record indicates that only *unwound, linear fragments of collagen type II* would be capable of being detected by the antibodies disclosed in the specification.

In particular, the postfiling literature provides evidence that the HRGYPGLDG sequence is not accessible to antibody binding in the context of the native protein, but rather is only detectable in *linear, unwound* fragments of collagen type II that no longer retain the triple helix structure of wound collagen. For example, Manicourt et al. (of record) teach that native, fully wound collagen is cleaved by proteases to yield denatured collagen, which exposes neoepitopes

that are normally hidden and therefore not recognized in the native triple helix (see page 425, right column, the last paragraph). Such neoepitopes include the Coll2-1-NO2 peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) that is disclosed in the instant specification (see Table 1 and page 428, left column). Manicourt et al. therefore teach that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of native, wound collagen type II, consistent with the data presented in the specification.

Applicant's postfiling work also identifies the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence as a neoepitope that is not present in either native or in heat-denatured type II collagen, but only in the context of linear fragments. See Deberg et al. (of record, especially p. 260, right column, "Antiserum specificity"; and Figure 1). For example, Deberg et al. teach that an antisera (D3) specific for the HRGYPGLDG epitope (termed "Coll 2-1") did not bind to heat-denatured type II collagen, "suggesting that D3 was *specific for the linear form* of Coll 2-1" (page 260, right column, the second to last paragraph, emphasis added).

Therefore, in describing only antibodies that recognize the neoepitope sequence HRGY(NO_2)PGLDG, Applicant has not described how to detect all "free fragment forms" that include this sequence, since only unwound, linear fragments present the neoepitope sequence. The claim term "free fragment" is not equivalent in scope to "unwound, linear fragment". Furthermore, the term "free" in the context of fragments has not been assigned a specific meaning according to the instant specification, where it is only mentioned in the context of distinguishing fragments from *full-length protein.* The specification does not disclose detection only of unwound, linear fragments. For these reasons, one skilled in the art cannot envisage

possession of the claimed methods of detecting all "free fragment" forms claimed, since it is now known that only unwound linear fragments present the epitope.

Scope of Enablement

19. Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for detecting the sequence consisting of HRGY(NO_2)PGLDG (SEQ ID NO:6) using context-dependent antibodies raised against SEQ ID NO:6 that do not bind to HRGYPGLDG (SEQ ID NO:1), does not reasonably provide enablement for methods of detecting **all** amino acid sequences that comprise the sequence HRGY(NO_2)PGLDG (SEQ ID NO:6) by any means, or for methods of detecting amino acid sequences comprising HRGYPDGDG (SEQ ID NO:1) and a nitrated tyrosine. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The nature of the invention relates to the production of antisera against peptides consisting of the sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), where Y(NO_2) is nitrotyrosine, and the use of such antisera in detection methods involving serum. See especially page 7, lines 23-27 and Examples 1, 5, and 6. The antisera were successfully used in methods of detecting the eliciting peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) (see Example 2).

The antisera had strong affinity to the nitrated sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), but only weak affinity to the non-nitrated sequence HRGYPGLDG (SEQ ID NO:1) (Example 1). This means that the epitope recognized by the antisera included the nitrotyrosine residue, such that the antisera discriminate the nitrated from the non-nitrated sequence.

The antisera produced against the peptides were seen to have no binding affinity to free L-nitrotyrosine (see Example 2, especially at page 23, lines 5-6), such that the antisera are *context-dependent*, i.e. they do not bind to free nitrotyrosine, but rather, only recognize the nitrotyrosine residue that appears in the context of SEQ ID NO:6. See the written description rejection above for further discussion of context-dependent vs. context-independent antibodies.

By contrast, the claims are broadly drawn to methods of detecting either (a) sequences comprising HRGY-NO_2-PGLDG or (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue at some other position in the sequence.

The specification discloses that HRGYPGLDG (SEQ ID NO:1) corresponds to a portion of the triple helical region of the protein (see page 12, lines 26-30). The postfiling literature provides evidence that the HRGYPGLDG sequence is not accessible to antibody binding in the context of the native protein, but rather is a "neoepitope" that is only detectable in linear, unwound fragments of collagen type II that no longer retain the triple helix structure of wound collagen.

In particular, Manicourt et al. (as discussed above) teach that native, fully wound collagen is cleaved by proteases to yield denatured collagen, which exposes neoepitopes that are normally hidden and therefore not recognized in the native triple helix (see page 425, right column, the last paragraph). Such neoepitopes include the Coll2-1-NO2 peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) that is disclosed in the instant specification (see Table 1 and page 428, left column). Manicourt et al. therefore teach that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of native, wound collagen type II, consistent with the data presented in the specification.

Applicant's postfiling work also identifies the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence as a neoepitope that is not present in either native or heat-denatured type II collagen, but only in the context of *linear* fragments. See Deberg et al., which teaches that an antisera (D3) specific for the HRGYPGLDG epitope ("Coll 2-1") did not bind to native or heat-denatured type II collagen, "suggesting that D3 was *specific for the linear form* of Coll 2-1" (page 260, right column, the second to last paragraph, emphasis added).

Therefore, in describing how to make and use antibodies that recognize the neoepitope sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), Applicant has failed to enable the skilled artisan to detect all "free" fragments comprising that sequence, since only unwound, linear fragments present the neoepitope sequence. The antibodies taught in the specification are *not* capable of recognizing native type II collagen (as also disclosed in the instant specification), and therefore, the specification fails to enable the skilled artisan to detect all amino acid sequences that comprise the sequence HRGYPGLDG (SEQ ID NO:6) since it fails to teach how to detect fragments that retain the wound triple-helix structure of collagen. In light of the postfiling literature, it is apparent that only unwound linear fragments of collagen type II that comprise the sequence would be able to be detected by the antibody of the invention.

Claims 2-4 refer to an antibody that is immunoreactive with nitrated tyrosine. The claims encompass antibodies that recognize nitrated tyrosine in both a context-dependent (claim 3) and in a context-independent manner (claim 4). However, the specification is not enabling for "context-independent" antibodies since these would by definition recognize nitrated tyrosine regardless of the surrounding protein sequence. As such, that they could not be used alone in

order to detect the *specific* protein sequences claimed, since they would bind to all nitrotyrosine-containing sequences irrespective of the surrounding protein sequence.

Specifically, the specification lacks guidance as to how *context-independent* antibodies that recognize nitrotyrosine in any context may be used to detect the specific amino acid sequences claimed, i.e. those that comprise HRGY-NO_2-PGLDG or HRGYPGLDG, since by definition, antibodies that are context-independent would bind to all nitrotyrosine-containing sequences, and thus would not specifically detect certain sequences.

In the case of the claimed methods detecting (a) sequences comprising HRGY-NO_2-PGLDG, Applicant has not described how a context-independent antibody that binds to all nitrated tyrosine residues could be used to detect sequences that include this specific epitope, since by definition, antibodies that are context-independent would bind to all nitrotyrosine-containing sequences, and thus would not *specifically* react with those sequences that comprise HRGY-NO_2-PGLDG.

Similarly, the specification lacks guidance as to how to detect amino acid sequences in free fragment form that (b) comprise the sequence HRGYPGLDG and a nitrated tyrosine residue (i.e., elsewhere in the sequence). Although a context-independent anti-nitrotyrosine antibody may bind to such sequences, it would also bind to all other nitrotyrosine-containing sequences. This would not result in preferential detection of amino acid sequences in free fragment form that comprise the sequence HRGYPGLDG and a nitrated tyrosine residue as claimed. See also the new matter and written description rejections above.

Even in the case of the two-antibody methods of claim 4, which involve a first context-independent antibody (e.g., anti-nitrotyrosine antibody) followed by a second antibody that is

specific for "an amino acid sequence which is specific for and uniquely identified with a specific protein", the claims broadly encompass a large genus of such "specific for and uniquely identified with" sequences, yet the specification fails to disclose any sequences or working examples in which such two-antibody methods were used to detect the claimed sequences.

In particular, in the case of detecting (a) sequences comprising HRGY-NO_2-PGLDG, because the claimed two-antibody methods involve the use of a first anti-nitrotyrosine antibody, this means that the first antibody would bind to the nitrotyrosine residue in the HRGY-NO_2-PGLDG sequence. Binding of the first antibody would therefore block this epitope, rendering it inaccessible for binding by a second antibody. Therefore, the second antibody would need to target another epitope; however, the only sequence that is disclosed with any particularity is the HRGYPGLDG sequence. The epitope to be targeted by the second antibody is not known. The specification does not disclose what other sequences would be present on those fragments that also contain HRGY-NO_2-PGLDG, such that in order to carry out the claimed invention, one skilled in the art would need to first (1) detect and identify all free fragment forms that include the HRGY-NO_2-PGLDG, (2) identify other epitopes that are both common to all such fragment forms and also are "specific for and uniquely identified with a specific protein". For these reasons, therefore provides insufficient guidance as to how to carry out the claimed invention.

Even if the second antibody epitope were known, it is unclear how an anti-nitrated tyrosine antibody and a second antibody to some other sequence could be used to *selectively* detect sequences comprising (a) HRGY-NO_2-PGLDG. Since in this case neither of the two antibodies binds specifically to the HRGY-NO_2-PGLDG sequence, there is no basis for selective detection of sequences that include HRGY-NO_2-PGLDG. The claimed assay would select for

sequences that contain the second epitope and nitrotyrosine, not for sequences that contain HRGY-NO2-PGLDG and nitrotyrosine.

Similarly, there is a lack of guidance as to how to detect (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue, since although the epitope recognized by the first antibody is recited (nitrated tyrosine), the second antibody epitope is not known. Further, even if the second antibody epitope were known, Applicant has not described how an anti-nitrated tyrosine antibody and a second antibody to another sequence could be used to detect those particular sequences that comprise HRGYPGLDG. Since in this case neither antibody is recited as binding to the HRGYPGLDG sequence, there is no basis for selective detection of sequences that include HRGYPGLDG. The claimed assay would select for sequences that contain the second epitope and nitrotyrosine, not for sequences that contain HRGYPGLDG and nitrotyrosine.

Turning to the use of "context-dependent" antibodies (as disclosed for example at page 5, lines 6-11 of the specification), it is noted that the specification discloses that the antisera of the invention discriminates between the nitrated and the non-nitrated form of the SEQ ID NO:6, since the antisera had only weak affinity to the non-nitrated sequence (see page 23, lines 1-6). However, the claims broadly recite only that the antibody is reactive with nitrated tyrosine (see claim 2). Claim 3 recites that the binding partner is "specifically reactive" with the nitrated form; however, since Applicant has not defined the term "specifically reactive", the claims do not exclude antibodies that react with **both** the nitrated and the non-nitrated tyrosine.

The claims fail to clearly recite that the antibodies distinguish between the nitrated HRGY-NO2-PGLDG and the non-nitrated HRGYPGLDG sequence. The specification fails to

teach the skilled artisan how to use antibodies that bind to both nitrated and non-nitrated sequences in methods of detecting the specific nitrated amino acid sequences claimed. There are no working examples in which such antibodies were employed.

Furthermore, Applicant has not provided any direction or guidance as to how to detect (b) sequences comprising HRGYPGLDG and a nitrated tyrosine residue using context-dependent antibodies as in claim 3. In this case, the context-dependent antibody is not binding to the HRGYPGLDG sequence since the nitrated tyrosine residue is present at some other, unspecified part of the sequence to be detected. However, Applicant is broadly claiming methods of using context-dependent antibodies but has not disclosed the context (i.e., the epitope). Neither the epitope recognized by the antibody nor any examples of such antibodies themselves have been disclosed. This is insufficient guidance to carry out the claimed invention. Even if one skilled in the art were to identify nitrated sequences that comprise HRGYPGLDG, identify residues that surround the nitrated tyrosine, make synthetic peptides that include the nitrated tyrosine and surrounding residues, and raise antibodies against the peptide, it is unclear how such an antibody could be used to detect the claimed sequences. There is no basis for selection of sequences comprising HRGYPGLDG and a nitrated tyrosine residue since the HRGYPGLDG sequence is not being targeted for binding.

The claims now recite detection of an amino acid sequence "in a free fragment form", but are not limited as to the type of detection method employed. In particular, claim 1 does not require the use of an antibody. However, the only reference made to detection of HRGYPGLDG in "free" fragments is presented in the Example on pages 29-30, in which it is stated that the D33

antiserum only recognizes the nitrosylated HRGY-NO2-PGLDG sequence "in the form of free fragments".

Therefore, there is a lack of direction and guidance to detection of sequences "in a free fragment form" by any other means except by use of a context-dependent antibody raised against the HRGY-NO2-PGLDG epitope. This teaching is not commensurate with the scope of the claims, which are not limited to methods using D33 antibody or even to methods that involve antibody detection. There is no disclosure at all relating to detection of sequences "in a free fragment form" by other detection methods that would be encompassed by the claim, e.g. HPLC.

Finally, it is noted that although claim 1 now is limited to detection of sequences that contain HRGYPGDLG, which is disclosed as being a portion of collagen type II. However, claims 3-7 refer to "an amino acid sequence which is specific for and uniquely identified with a specific protein", such that the claims apparently continue to broadly encompass detection of HRGYPGDLG-containing sequences in other proteins. For example, claim 7 refers to cartilage link protein and other proteins in addition to collagen type II. Since the prior art fails to teach that HRGYPGLDG is found in any of these other proteins, the specification fails to teach how to detect HRGYPGLDG-containing sequences that are "specific to and uniquely identified with" cartilage link protein or aggrecan, for example.

Applicant is reminded that should the claims be amended in accordance with the scope of enabling disclosure indicated above, any amendments must be supported by the original disclosure and must also comply with the requirements of 35 USC 112, 2nd paragraph.

20. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

21. Claims 2-7 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

22. Claims 2-4 recite the limitation "**said nitrated tyrosine**". There is insufficient antecedent basis for this limitation in the claims because claim 1 refers to two different nitrated tyrosine residues (see lines 4 and 5-6); thus, there is ambiguity as to which of the nitrated tyrosines is being invoked by reference to "said nitrated tyrosine".

23. Claims 3 and 5 recite the limitation "**said amino acid sequence**". There is insufficient antecedent basis for this limitation in the claims because claim 1 refers two different amino acid sequences (see lines 2-3 and 4-5); thus, there is ambiguity as to which amino acid sequence is being referred to.

24. Claim 3 recites that the antibody "**is specifically reactive with said nitrated tyrosine in the context that said amino acid sequence is specific to and uniquely identified with a specific protein**". This wording is vague and indefinite and the meaning cannot be ascertained. In particular, it is not understood what is meant by an antibody that is reactive "in the context that said amino acid sequence...".

25. Claims 3-5 refer to an amino acid sequence which is "**specific to and uniquely identified with a specific protein**", which is vague and indefinite. This terminology does not appear and is not defined in the specification. It is unclear what types of amino acid sequences would be considered to be "specific to and uniquely identified with" a specific protein since no limiting definition is given. For example, would this term encompass a given sequence that is

only known to be found in one specific protein, or alternatively, would the term include sequence motifs that are common to a family of proteins? Alternatively, the term could be interpreted as meaning that the sequence is simply known to be present in a given protein. In the absence of a specific definition for the term in this context, the metes and bounds of the claim are unclear.

26. Claim 7 recites that the amino acid sequence that is detected is a sequence that is specific to and uniquely identified with "collagen of type I, II, III, VI, IX or XI, aggrecan, cartilage link protein, cartilage oligomeric protein, or cartilage intermediate layer protein". However, claim 1 now specifies that the detected amino acid sequence comprises HRGYPGLDG (SEQ ID NO: 1). It is unclear how this sequence, which is disclosed to be a fragment of collagen type II (Example 1), could also be considered to be "uniquely identified" with these numerous other proteins. There is nothing of record to indicate that the sequence is found in any other protein except collagen type II. Furthermore, it is unclear in general how a single sequence could be said to be "uniquely" identified with several specific proteins.

Response to Arguments

27. Applicant's arguments filed 6/4/07 have been fully considered.

28. With respect to the rejections of claims 1-9 under 35 USC 112, 1st paragraph (written description and scope of enablement), Applicant's Reply does not present specific arguments traversing the rejections but states that the rejections have been overcome by the instant amendments (Reply, page 6), to which the Examiner disagrees for the reasons set forth above in the rejections.

Conclusion

29. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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